

From the Department of Medicine Solna

Karolinska Institutet, Stockholm, Sweden

IN SITU CHARACTERIZATION OF IMMUNE CELLS MEDIATING POTENTIAL CONTROL OF HIV INFECTION IN THE FEMALE GENITAL MUCOSA

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**Karolinska
Institutet**

Stockholm 2016

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Published by Karolinska Institutet.

Printed by E-Print AB 2016

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ISBN 978-91-7676-258-5

In situ characterization of immune cells mediating potential control of HIV infection in the female genital mucosa

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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To my family

ABSTRACT

Heterosexual HIV transmission is the most common viral transmission route, worldwide, where young women are more susceptible to HIV infection than men. To establish a persistent infection the virus needs to cross the mucosal surface of the female genital tract (FGT). The FGT mucosa is thus considered to be the portal of HIV entry and initial site of viral replication. A better understanding of the immunological milieu at the portal of viral entry is crucial for the development of preventive interventions. Hence, in this thesis we quantified and characterized immune cells, primarily subsets of CD8⁺ T cell, in the FGT tissues of HIV-infected and uninfected women.

In **Paper I** and **II**, we observed that HIV-infected women displayed similar levels of CD4 expression in their ectocervix as compared to uninfected controls. However, they showed higher immune activation levels as well as signs of HIV replication in their cervix. An inflammatory environment in the genital mucosa may promote viral replication and genital shedding and thereby increase the risk of sexual HIV transmission. The HIV-infected women also had elevated levels of cervical CD8⁺ cells, which correlated significantly with cervical viral load. The CD8⁺ cells were predominantly intraepithelial CD8⁺ T cells and 60% of them expressed CD103 (**Paper II, III**). Our data thus suggests that CD8⁺ T cells, including tissue-resident CD8⁺ T cells, may be actively recruited to/or expanded in the genital mucosa of chronically HIV-infected sex workers. This implies that tissue-residing CD8⁺ T cells play an important role in local HIV pathogenesis. To further investigate immune responses in the FGT mucosa we assessed the expression of MAIT cells in the genital tissues of healthy women (**Paper IV**). FGT-derived MAIT cells preferentially localized in the ectocervical epithelium and were biased towards IL-17 and IL-22 production upon bacterial stimulation. This indicates that functional MAIT cells localized near the luminal surface of the genital mucosa may be important for the preservation of the genital barrier integrity and may act as a first line of defence against invading pathogens.

In summary, this thesis describes the localization and distribution of immune cells in the genital mucosa of HIV-infected and uninfected women. Studies described here may contribute to the knowledge needed for development of vaccination and/or microbicide strategies against HIV and other sexually transmitted infections.

LIST OF SCIENTIFIC PAPERS

- I. **Stable CD4 expression and local immune activation in the ectocervical mucosa of HIV- infected women**
Taha Hirbod, Joshua Kimani, Annelie Tjernlund, Juliana Cheruiyot, ANNA PETROVA (GIBBS), Terry B. Ball, Nelly Mugo, Walter Jaoko, Francis A. Plummer, Rupert Kaul and Kristina Broliden
Journal of Immunology. 2013 Oct 1; 191(7):3948-54.
- II. **Presence of CD8⁺ T cells in the ectocervical mucosa correlates with genital viral shedding in HIV-infected women despite a low prevalence of HIV RNA-expressing cells in the tissue**
ANNA GIBBS, Taha Hirbod, Qingsheng Li, Karin Bohman, Terry B. Ball, Francis A. Plummer, Rupert Kaul, Joshua Kimani, Kristina Broliden and Annelie Tjernlund
Journal of Immunology. 2014 Apr 15; 192(8):3947-57.
- III. **HIV-infected women display lower proportion of cervical and circulating CD103⁺ cells among CD8⁺ T cells, despite the overall increase in total numbers of tissue-resident CD8⁺ T cells in their cervical epithelium**
ANNA GIBBS, Marcus Buggert, Petter Ranefall, Andrea Introini, Stanley Cheuk, Liv Eidsmo, Taha Hirbod, Terry B. Ball, Joshua Kimani, Rupert Kaul, Annika C. Karlsson, Carolina Wählby, Kristina Broliden and Annelie Tjernlund
Manuscript.
- IV. **MAIT cells reside in the female genital mucosa and are biased towards IL-17 and IL-22 production in response to bacterial stimulation**
ANNA GIBBS, Edwin Leeansyah, Andrea Introini, Dominic Paquin-Proulx, Klara Hasselrot, Emilia Andersson, Kristina Broliden, Johan K. Sandberg and Annelie Tjernlund
Accepted in **Mucosal Immunology**.

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LIST OF ABBREVIATIONS

AIDS	Acquired immune deficiency syndrome
APC	Antigen presenting cell
APOBEC	Apolipoprotein B mRNA editing enzyme catalytic, polypeptide-like
ART	Antiretroviral therapy
BV	Bacterial vaginosis
CCR	CC chemokine receptor
CD	Cluster of differentiation
CTL	Cytotoxic T lymphocytes
CVS	Cervicovaginal secretions
CVL	Cervicovaginal lavage
CXCR	CXC chemokine receptor
DC	Dendritic cell
DC-SIGN	DC-specific ICAM-3 grabbing non-integrin
DNA	Deoxyribonucleic acid
Eomes	Eomesodermin
FGT	Female genital tract
FSW	Female sex workers
HAART	Highly active antiretroviral therapy
HESN	HIV-exposed seronegative
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HPV	Human papillomavirus
HSV	Herpes simplex virus
Ig	Immunoglobulin
IFN	Interferon
IL	Interleukin
LPS	Lipopolysaccharides
MHC	Major histocompatibility complex
MNC	Mononuclear cells
MR	Mannose receptor
NHP	Non-human primate
NK	Natural killer
PBMC	Peripheral blood mononuclear cell
PCR	Polymerase chain reaction
PD-1	Programmed death-1
PLZF	Promyelocytic leukemia zinc finger

PrEP	Pre-Exposure Prophylaxis
PRR	Pattern recognition receptor
PVL	Plasma viral load
ROR γ t	Retinoic acid-related orphan receptor γ t
RNA	Ribonucleic acid
SIV	Simian Immunodeficiency virus
STI	Sexually transmitted infection
TCR	T cell receptor
TLR	Toll-like receptor
TNF	Tumour necrosis factor
TRIM	Tripartite Motif
TNF	Tumour necrosis factor

1 INTRODUCTION

1.1. The human immunodeficiency virus (HIV)

The year 1981 is generally referred to as the beginning of the human immunodeficiency virus type 1 (HIV-1) epidemic. In 1981, the Centre for Disease Control reported about five, previously healthy, homosexual men who had developed *Pneumocystis pneumonia*, a disease normally associated with severe immunosuppression.¹ Shortly after, other cases of rare opportunistic diseases together with symptoms including fever and weight loss become evident, particularly in homosexual men.^{2,3} The unknown disease emerging in these individuals was characterized by severe immunosuppression, and it was therefore named acquired immunodeficiency syndrome (AIDS).⁴ In 1983, a group of French scientists identified the causative agent of AIDS to be a virus that was later named HIV-1.⁵ After the discovery of HIV-1, a second type of the virus (HIV-2) was isolated from AIDS patients originated from West Africa.⁶ However, it is HIV-1 that is almost entirely responsible for the HIV epidemic worldwide and it will hence be discussed in this thesis and referred to as HIV unless otherwise stated.

HIV originates from the related Simian Immunodeficiency virus (SIV), which infects non-human primates (NHP).^{7,8} The outcome of the SIV disease progression varies in the natural (sooty mangabeys, African green monkeys and others) and non-natural hosts (such as monkeys of Asian origin). Namely, the natural SIV-infected hosts do not develop a clinical disease (e.g. these monkeys resemble an AIDS-resistant phenotype), while in the non-natural hosts, SIV infection causes a disease similar to human AIDS.⁹ The SIV-infected NHP provide a valuable animal model to study HIV related pathogenesis and evaluate diverse prophylactic and therapeutic approaches.

1.1.2. HIV viral structure and life cycle

HIV can be spread by sexual contact, from infected mother to her child and as a blood-borne infection. HIV is a member of the genus *Lentivirus* and belongs to the family of retroviruses (*Retroviridae*). Like other lentiviruses, HIV is capable of establishing a long-term slowly progressive disease. HIV is an enveloped virus containing two copies of positive-stranded ribonucleic acid (RNA), packaged within a core of viral proteins. The HIV RNA genome is about 9.2 kb and consists of long terminal repeats and genes encoding for structural proteins (*gag*), envelope glycoproteins (*env*), viral enzymes (*pol*), regulatory proteins (*tat*, *rev*) and accessory proteins (*vif*, *nef*, *vpr*, *vpu*).

HIV target cell entry occurs through the viral envelop protein gp120, which interacts with the major receptor cluster of differentiation (CD)4 and with one of the two co-receptors (CC chemokine receptor (CCR)5 and CXC chemokine receptor (CXCR)4) (**Figure 1**). However, the majority of initial HIV transmissions occur in a CCR5 dependent manner.¹⁰ Moreover, Caucasian individuals who have a 32 base pair deletion within the CCR5 gene (*CCR5Δ32*)

display a protective phenotype against HIV infection,¹¹ indicating that the CCR5 co-receptor plays a crucial role for the establishment of the viral infection. After successful entry, viral material is released into the host cell where the reverse transcriptase transcribes one of the viral RNA copies into a double-stranded deoxyribonucleic acid (DNA) that is further transported into the nucleus. At the same time, the viral enzyme integrase enters the nucleus and initiates the integration of viral DNA into the host cell genome. The integrated viral DNA can remain transcriptionally silent for many years and thereby establish viral latency. Upon active transcription, the viral mRNA is subsequently translated near the endoplasmic reticulum. Newly synthesized viral proteins are further assembled into a viral particle and released by budding from the cellular membrane.

HIV displays a great replicative capacity. It has been estimated that up to 10^8 cells can be infected, and more than 10^{10} viral particles can be produced daily.^{12,13} Moreover, HIV replication is associated with a high mutation rate that enables the virus to evade immune responses, develop resistance to pharmacological treatments, and makes it difficult to develop an effective vaccine.

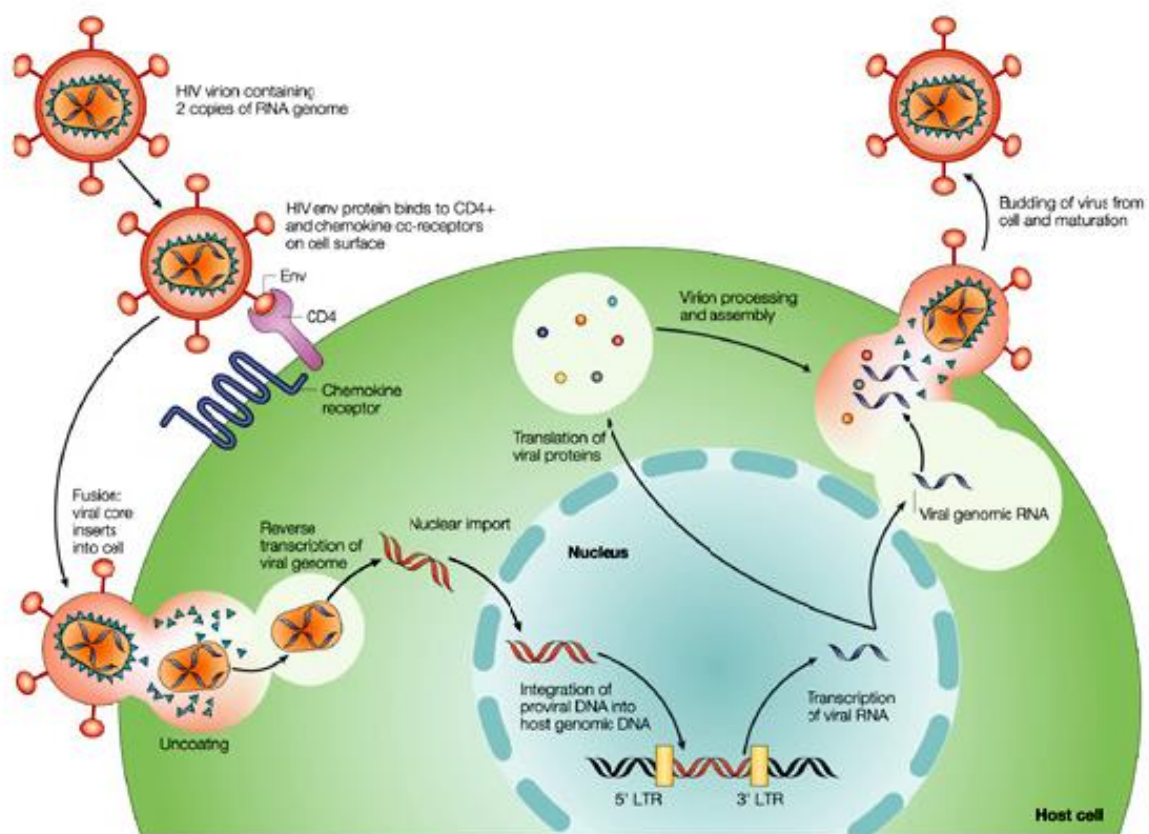


Figure 1. HIV life cycle. Schematic picture showing that HIV goes through multiple steps (Binding and Fusion; Reverse Transcription; Transcription; Assembly; Budding and Maturation) to reproduce itself and create more virus particles. Reprinted with permission.¹⁴

1.2. Global HIV distribution

By the end of 2014, around 40 million people were reported to be HIV infected. During 2014 about 2 million people became newly infected with HIV and around 1.2 million people died from AIDS related illnesses.¹⁵ African countries bear the burden of the HIV epidemic, with heterosexual transmission being the most prevalent mode of HIV transmission (discussed below). Sub-Saharan Africa is considered to be the most affected region, with 66% of new HIV infections, globally, occurring in this region. In 2014, there were recorded 25.8 million people living with HIV in Sub-Saharan Africa and more than 70% of adults living in this region have never been tested for HIV.^{15,16} The rate of new HIV infections has increased by 26% in the Middle East and North African region during the past 14-15 years. Alarming facts are furthermore coming from Eastern Europe and Central Asia with 140,000 new infections were estimated in 2014, which was 30% higher as compared to year 2000.¹⁵

Despite the availability of antiretroviral therapy (ART) and comprehensive public health measures, HIV transmission in the European region has not been drastically declining. In Western Europe, sex between men is the predominant mode of HIV transmission in contrast to Eastern Europe where transmission through drug injections and heterosexual transmission are the most common transmission routes.¹⁷

1.2.1. HIV prevalence among key populations: female sex workers

It is evident that HIV prevalence among sex workers is 12 times higher than among the general population.¹⁸

In Kenya, HIV prevalence among the general population is around 6%. However, among key populations, such as men who have sex with men and female sex workers (FSW), HIV prevalence is considerably higher. FSW have the highest reported HIV prevalence (30%) among other groups in Kenya.¹⁹ Furthermore, it has been shown that about 50% of HIV-positive FSW are unaware of their HIV infection. As a consequence, FSW continue to pose high risk of HIV transmission as well as experience delays in access to treatment and healthcare.²⁰ Despite high ART coverage among the general Kenyan population, with >80% of ART-eligible people on treatment, only about 20-30% of HIV-infected FSW are receiving ART.^{19,21}

Unprotected sex, sexual violence, discrimination as well as social and economic factors are associated with the increased vulnerability to HIV transmission in this group.¹⁸ It is suggested that elimination of sexual violence alone could prevent 17% of HIV infections in FSW and their clients. Moreover, decriminalization of the sex work could avert 33% of HIV infections in FSW and their clients in the next decade.²¹ This indicates that assessment of the issues present in key populations together with legal measures may reduce HIV transmission in these groups and subsequently overall HIV transmission.

1.2.2. HIV today

The introduction of ART can truly be considered as one of the major achievements on the way of defeating the HIV epidemic. The introduction of ART has changed the perception of HIV as a deadly disease to a manageable chronic condition. When ART was shown to reduce HIV transmission,²² HIV treatment or Pre-Exposure Prophylaxis (PrEP) was implemented as a prevention measure for individuals at high risk of HIV infection.²³ Today, new ambitious treatment goals are set to end the HIV epidemic. To achieve the 90-90-90 treatment target by year 2020, i.e. 90% of people living with HIV will know their HIV status, 90% of people living with HIV who know their status will receive ART, and 90% of people on treatment will have suppressed viral loads (90-90-90). And most importantly, to reach the ultimate goal of ending the HIV epidemic by year 2030.²⁴

In 2001, only 1 million people living with HIV had access to ART, but in 2015, more than 15 million people received antiviral treatment. Because of wide ART coverage the annual new infections dropped from 3.1 to 2 million, and the number of AIDS related deaths has dropped from 2 to 1.2 million in the past 15 years. However, as more individuals are gaining access to ART, the total cost increases, making wide ART access a big economical challenge. According to the UNAIDS estimates, \$31.1 billion will be required for the AIDS response in year 2020.¹⁶ Moreover, other issues associated with ART, including side effects, antiviral drug resistance and sub-optimal antiviral drug penetration in viral reservoirs, remain unresolved.^{25,26}

1.3. The immunopathogenesis caused by HIV infection

As a virus that causes immunodeficiency syndrome, HIV infection deteriorates virtually all components of the immune system. HIV associated alterations are especially pronounced in the T cell compartment and result in increased activation of the immune system.

1.3.1. CD4⁺ T cells

Progressive CD4⁺ T cell depletion is a clinical manifestation of HIV infection and CD4⁺ T cell count is the most common marker used for monitoring the progression of HIV disease. CD4⁺ T cells are the preferential target cells for HIV infection and hence these cells are primarily affected by HIV pathogenesis. The initial CD4⁺ T cell destruction occurs due to the direct cytopathic effects caused by the virus.²⁷ During the early phase of HIV infection, massive depletion of CD4⁺ T cells occurs in mucosal compartments (mainly in the gastrointestinal mucosa) as well as in peripheral blood and lymph nodes.²⁸ Likewise, the depletion of cervical CD4⁺ T cells is observed in early HIV infection.²⁹ A progressive CD4⁺ T cell homeostatic failure, mediated by the virus itself, and together with the high immune activation ultimately leads to the cell depletion over time.²⁷ Dramatically low levels of CD4⁺ T cells, <200 cells/mm³, are associated with HIV progression to AIDS.

While early ART initiation effectively restores the CD4⁺ T cell levels, they do not reach the same magnitude as present in HIV-uninfected individuals.³⁰

Additionally, latently infected resting memory CD4⁺ T cells constitute the main viral reservoir, in both ART- treated and treatment naïve patients,³¹ and this is considered to be one of the major obstacles for successful HIV eradication.³²

1.3.2. CD8⁺ T cells

While the increased magnitude of HIV-specific CD8⁺ T cell responses is associated with the drop of acute viremia, already during the early stage of the infection these cells become inefficient and lose their ability to efficiently control HIV replication.³³ The expansion of the HIV-specific CD8⁺ T cell pool peaks at median of 250 days post infection and remains elevated during the course of the infection.^{34,35} However, the HIV-specific CD8⁺ T cells represent less than 10% of the total CD8⁺ T cell pool, with the rest accounting for reactivated responses to latent pathogens, such as herpes viruses, and bystander activated CD8⁺ T cells.³⁵ Moreover, both HIV-specific and bulk CD8⁺ T cells display a highly activated phenotype, which is often defined by the upregulation of CD38 and human leukocyte antigen (HLA)-DR molecules.^{36,37} Particular expansion of CD38 and HLA-DR expressing CD8⁺ T cells has been shown to be associated with decreased levels of CD4⁺ T cells and progression of AIDS.³⁶ Persistent antigenic exposure subsequently leads to the exhaustion of CD8⁺ T cells and progressive loss of their functions.³⁵ Expression of exhaustion markers, such as programmed death-1 (PD-1), and inhibitory molecules on HIV-specific CD8⁺ T cells is associated with poor polyfunctionality of these cells and positively correlates with the plasma viral load (PVL).³⁸⁻⁴⁰ Moreover, HIV-specific CD8⁺ T cells isolated from blood of chronic HIV-infected patients have been shown to display poor cytolytic capacity.⁴¹ T-box transcription factors T-bet and Eomesodermin (Eomes) are transcription factors that have a crucial role in regulating the effector and memory differentiation of CD8⁺ T cells.⁴² Imbalance between the T-bet and Eomes expression in HIV-specific CD8⁺ T cells (T-bet^{dim}Eomes^{high}) has been associated with elevated expression of inhibitory molecules, poor polyfunctionality and decreased expression of Granzyme B as compared to corresponding T cells expressing higher levels of T-bet and lower levels of Eomes.⁴⁰ Concurrently, in HIV- infected individuals, CD8⁺ T cells accumulate within the intermediate memory pool and exhibit a skewed maturation phenotype.⁴³ Altogether this suggests that expansion of activated/exhausted early differentiated and poorly functional HIV-specific CD8⁺ T cells fails to control the viral replication. Furthermore, even the administration of ART fails to completely restore the elevated CD8⁺ T cell numbers, thus resulting in a low CD4/CD8 ratio in HIV-infected individuals.⁴⁴ Low CD4/CD8 ratio has been observed in ART-treated individuals experiencing AIDS unrelated morbidities,⁴⁵ suggesting that despite the effective treatment, the immunological imbalance may lead to the non-AIDS related adverse clinical events.

1.3.3. Immune activation

Immune activation and inflammation is a hallmark of HIV infection. Levels of immune activation are directly associated and predictive of HIV disease progression.^{36,46} Apart from the highly activated T cells, HIV pathogenesis also induces activation of B cells and of cells belonging to the innate immune system.^{47,48} Furthermore, elevated levels of proinflammatory cytokines such as type-1 interferons (IFN), tumour necrosis factor (TNF) α , interleukin (IL) 1 β and IL-6 are observed in circulation and mucosal compartments of HIV-infected individuals.^{46,49} HIV pathogenesis is also associated with disruption of barrier integrity in mucosal tissues.^{50,51} Compromised integrity of the mucosal barrier in the intestinal tract (“leaky gut”) subsequently leads to the translocation of the microbial products from the gut into the circulation. Increased levels of plasma Lipopolysaccharides (LPS), a surrogate marker of microbial translocation, directly correlates with the activated state of the immune system.⁵²

Moreover, persistent immune activation is likely to cause a systemic ageing of physiological functions.⁴⁶ Namely, HIV infected individuals present similarities with individuals of old age, and more often experience age-related diseases compared to age-matched uninfected adults.⁵³ Altered physiological functions and immunological abnormalities persist even after the ART treatment,⁵³ indicating that even treated HIV-infected individuals do not fully restore back to good general health conditions.

1.4. Heterosexual HIV transmission

The majority of all HIV transmissions, worldwide, occur through heterosexual transmission, where young women are twice as likely to become infected with HIV as compared to men.⁵⁴ Women represent about half of all people living with HIV, and while the incidence of HIV is declining globally, its impact disproportionally concerns young women. According to the 2015 UNAIDS report, the majority of newly HIV-infected are represented by young women; e.g. about 62% among 15-19 years old, and 56% among 15-24 years old.¹⁶ This “feminization” of the HIV epidemic is particularly pronounced in Sub-Saharan Africa where women account for two out of three new HIV infections.⁵⁵

All sexual transmissions occur across a mucosal surface and the genital mucosa is therefore considered to be the portal of viral entry and the first site where HIV replication occurs. However, the efficiency of vaginal male- to- female HIV-1 transmission is low, about 0.08-0.3 % per unprotected sexual intercourse,⁵⁶ which indicates the effectiveness of the genital barrier against HIV. Recent data indicate that establishment of viral infection via the heterosexual mode of transmission is associated with genetic background of the transmitted virus, that favours increased viral fitness. Furthermore, selection bias of the transmitted virus is lower in a more permissive environment, e.g. presence of the genital inflammation, but also in the virus transmitted to women, compared to men, suggesting a more permissive environment in the female genital mucosa.⁵⁷

1.5. The female genital tract

The female genital tract (FGT) consists of different compartments, responsible for two major tasks, providing a protection against invading pathogens and at the same time creating a tolerogenic environment for semi-allogenic fetus.^{55,58} The FGT can be divided in distinct anatomical regions; upper (ovaries, fallopian tubes, uterus) and lower (cervix and vagina) FGT (**Figure 2**). The mucosa of the uterus (endometrium) consists of a monolayer of columnar epithelial cells, and undergo major structural and functional changes during the course of the menstrual cycle.⁵⁵

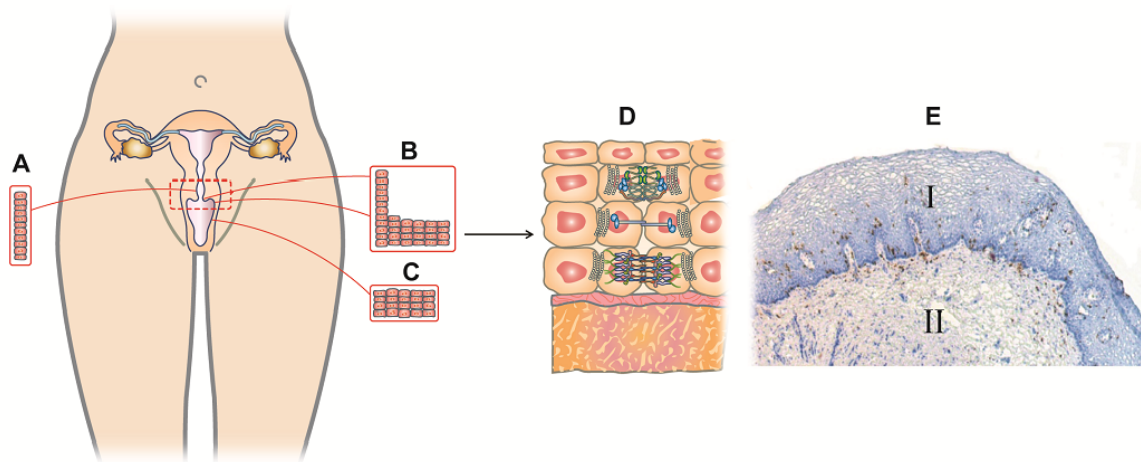


Figure 2. Schematic representation of the female genital tract. **A)** Single layer of the columnar epithelium in the endometrium and endocervix. **B)** Transformation zone between the endo- and the ectocervix. **C)** Multilayer squamous epithelium in the ectocervix and vagina. **D)** Distribution of tight junction proteins within the multilayered epithelium. **E)** Distribution of CD4⁺ cells (brown) in the **I.**) epithelial and **II.)** submucosal compartments of the ectocervix. The ectocervical tissue section was counterstained with hematoxylin (blue) to visualise all cells.

The cervical cavity (endocervix) connecting the uterus and vagina is covered by a single-layer of the simple columnar epithelial cells that produce endocervical mucus, providing an additional physical and antimicrobial barrier.^{59,60} The intravaginal portion of the cervix is called ectocervix and it is, just like the vagina, lined by a thick multilayer squamous epithelium. Intact ectocervical and vaginal epithelium provides a robust physical barrier, which harbours numerous immune cells and tight junction proteins.⁶¹⁻⁶³

The transition area between the endo- and the ectocervix, where the epithelium transforms from a single- to a multilayer, is called the transformation zone. This area is characterized by a particular abundance of immune cells (including HIV target cells) in a relatively small area, as compared to the rest of the FGT, suggesting an increased vulnerability at this site.^{55,64}

1.5.1. HIV target cells in the genital mucosa

HIV transmission can occur through the mucosal barriers in both the upper and the lower FGT.⁶⁵ However, the vaginal and the ectocervical mucosa display a larger surface area and are thus likely to be the primary sites to encounter transmitted virus during sexual intercourse^{61,65} (**Figure 3**). CD4⁺ T cells have been shown, in both NHP models and in cervical *ex vivo* explant models, to be the primary target cells for HIV infection.^{66,67} CD4⁺ T cells are abundant in ectocervical mucosa where they are dispersed in the epithelium and the underlying submucosa.⁶⁸ Recent studies indicate that FGT-derived IL-17 producing CD4⁺ T cells (Th17) as well as $\alpha 4\beta 7$ - and $\alpha 4\beta 1$ integrin expressing CD4⁺ T cells are relatively more susceptible to HIV infection as compared to other CD4⁺ T cell subsets.^{69,70} Furthermore, cervical Th17 cell are depleted early during the HIV infection, supporting the finding of the preferential infection of these cells.²⁹

Additionally, other cell types, including CD4 and CCR5 expressing dendritic cells (DCs) and macrophages, which are present in the female genital mucosa, are implicated in HIV susceptibility and transmission.^{71,72} *Ex vivo* studies in cervical explant models have shown that HIV virions can penetrate up to 50 μ m of the intact ectocervical epithelium.⁷³ Langerhans cells, a subset of DCs, residing in the ectocervical epithelium, are likely to be among the first cells to encounter virus and they can further pass the infectious virions to susceptible CD4⁺ T cells.^{68,74} They can either bind HIV through Langerin or via the CD4/CCR5 receptor complex.⁷⁵ Furthermore, submucosal DCs are also susceptible to HIV infection and can thus also facilitate CD4⁺ T cell infection, through the C-type lectin receptors, DC-specific ICAM-3 grabbing non-integrin (DC-SIGN) and mannose receptor (MR), and their interactions with gp120.⁷⁶⁻⁷⁸ CD4⁺CCR5⁺ macrophages are another cell population of HIV target cells that reside in the female genital mucosa and have been shown to be productively infected by HIV *ex vivo*.⁷⁹ After the establishment of the small focally infected population, also called “founder population”, the virus then disseminates to secondary lymphoid organs, which subsequently leads to systemic infection.

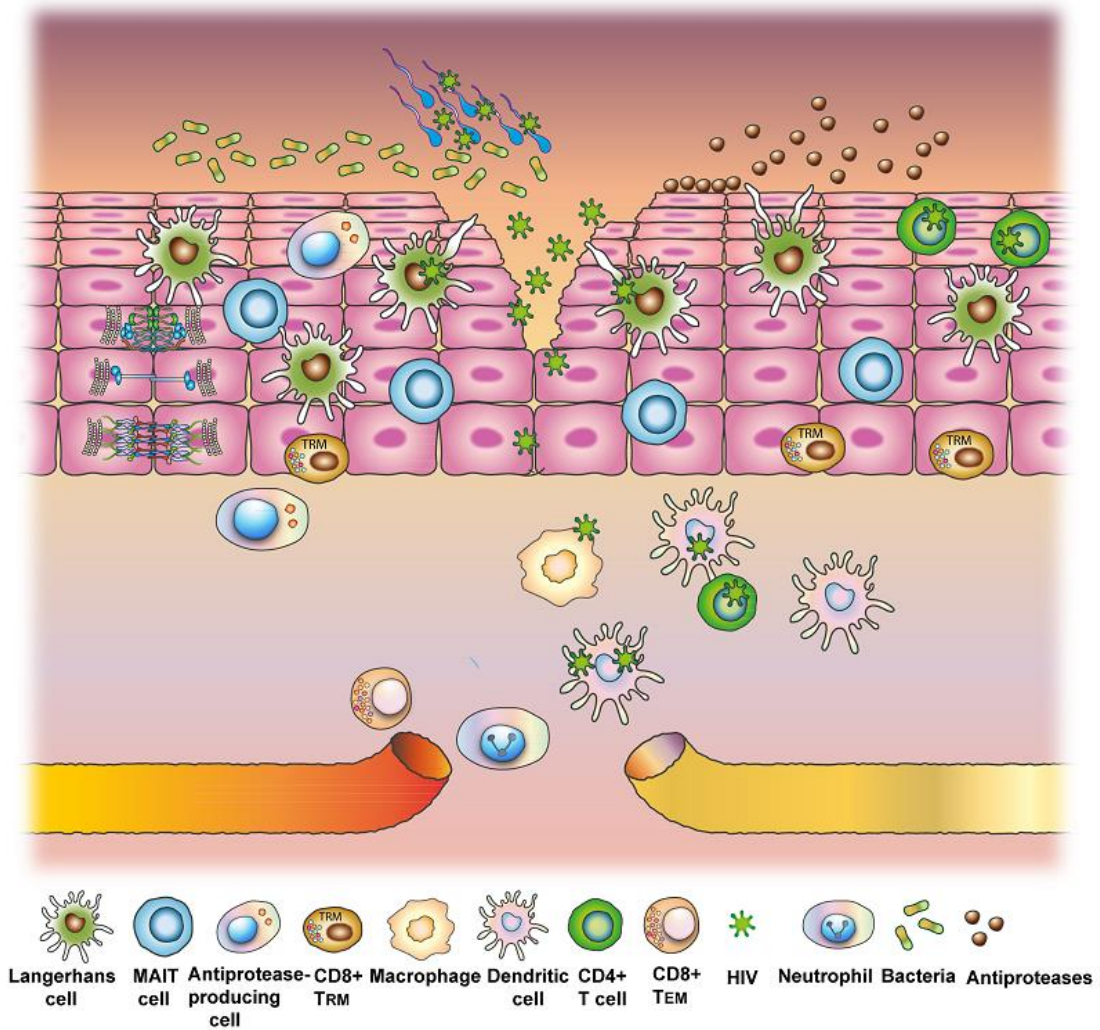


Figure 3. Schematic representation of the suggested mechanism for HIV heterosexual transmission across the female genital mucosa. The cervix and vagina are covered by a multilayered epithelium harboring HIV target and effector cells, as well as epithelial tight junction proteins, which are protecting against invading pathogens like HIV. The epithelial linings are covered by genital secretions containing hormones, microbes and innate proteins that also play an important role in HIV transmission. Microlesions in the epithelium are thought to facilitate entrance of HIV.

Despite the abundance of susceptible target cells in the female genital mucosa, the initial transmission events are associated with a relatively small founder population of infected cells. Only 40-50 SIV RNA⁺ cells have been detected in the endocervical mucosa after 3-4 days of SIV infection in NHP.^{66,80,81} Deep sequencing has furthermore revealed that in about 80% of cases of heterosexual HIV transmission, the initial infection is established by single virus genotype in a CCR5 dependent manner.¹⁰ Given the small size of the genetically homogeneous founder population, these initial events of HIV transmission represent great vulnerability of the virus and may therefore provide a good window of opportunity for preventive interventions (**Figure 4**).

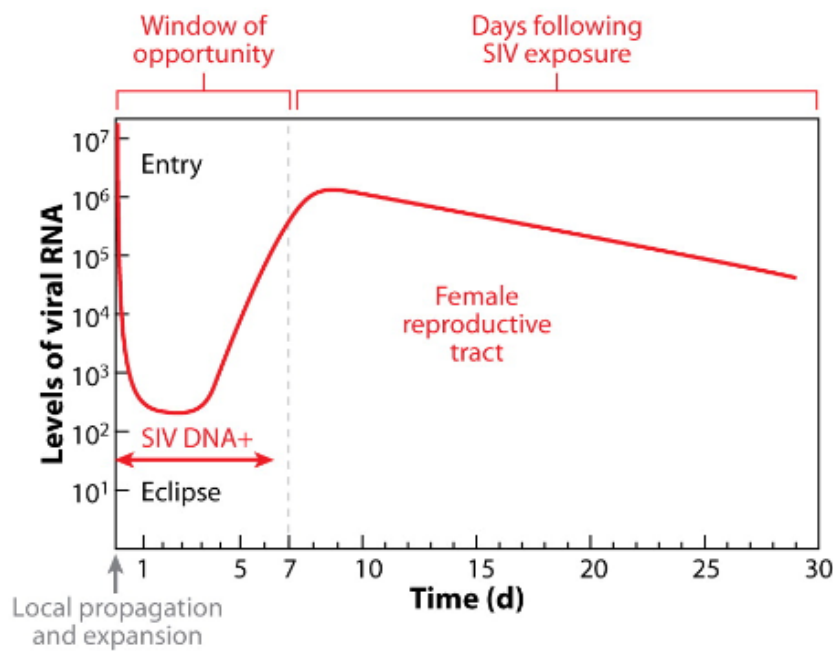


Figure 4. Window of opportunity. A schematic picture showing changes in the levels of SIV RNA in the FGT upon experimental SIV infection. The “window of opportunity” represents vulnerability of the virus and thus provides an opportunity for potential interventions. Adapted with permission.⁸²

1.5.2. Mucosal inflammation and immune activation

The strongest predictor of HIV transmission risk through sexual intercourse is viral load in blood and genital secretions of the HIV-infected partner.⁸³ Additionally, number of factors associated with inflammation and activation in the genital mucosa has been shown to subsequently increase sexual HIV transmission. Sexual activity *per se* as well as exposure to seminal fluid is associated with local inflammation and migration of activated lymphocytes to the genital tissues.⁸⁴ Elevated numbers of T cells, DCs and macrophages as well as higher levels of proinflammatory cytokines have been seen in the ectocervical tissues of women having unprotected sexual intercourse.⁸⁵ Presence of sexually transmitted infections (STIs), including, Herpes simplex virus (HSV)2, *N. gonorrhoeae*, *Chlamydia trichomonas* among others, contribute to a proinflammatory milieu in the genital mucosa and these STIs have also been associated with increased risk of HIV acquisition and transmission.⁸⁶⁻⁸⁸

Moreover, alterations in the local microflora of the FGT have also been associated with HIV susceptibility. While the dominance of *Lactobacillus* species in the genital microflora has been associated with lower HIV prevalence, dysbiotic women with clinical Bacterial vaginosis (BV) are at higher risk of HIV acquisition.^{89,90} Presence of BV in the genital mucosa is associated with reduced levels of factors involved in mucosal barrier integrity as well as elevated levels of inflammatory cytokines, which may partially explain why BV contributes to increased HIV acquisition.⁸⁴

Hormonal status has a great impact on the immunological environment in the FGT mucosa and thus, sex hormones and the menstrual cycle are believed to play a role in HIV susceptibility.⁵⁵ The progesterone-dominated luteal phase, rather than follicular phase, has been related to increased SIV transmission in NHP.⁹¹ Moreover, use of Depo-Provera, an injectable progesterone-based hormonal contraceptive, is associated with elevated levels of genital proinflammatory cytokines and increased risk of HIV of acquisition.^{87,92}

Conversely, low immune activation or a so-called “immune quiescent” state in the genital mucosa has been observed in HIV-exposed seronegative (HESN) individuals, who display a semi-resistant phenotype against HIV infection.⁹³ These HESN individuals are found in different cohorts, including HIV-seronegative commercial female sex workers from the Pumwani cohort, established in Nairobi, Kenya.⁹⁴ These individuals will be further discussed in this thesis and referred to as HIV-seronegative female sex workers (HIV-FSW).

Overall, the inflammatory environment and influx of HIV target cells to the genital mucosa can contribute to a higher risk of HIV acquisition as well as spark local viral replication and increase the transmission risk upon an ongoing infection.

1.6. Mucosal immune responses to HIV

1.6.1. Innate immunity

Innate immune responses represent the first line of defence and are often referred to as “non-specific immunity”. Components of the innate immunity provide rapid defence and usually act within hours upon encounter with the antigen. Moreover, production of chemokines and proinflammatory cytokines by innate cells, results in recruitment and activation of cells belonging to the adaptive, or the so-called “specific immunity”.⁹⁵

Recognition of foreign antigens by innate immune cells occurs through the pattern recognition receptors (PRRs). Various PRRs, including Toll-like receptors (TLRs) expressed on innate immune cells can trigger inflammatory cascade resulting in induction of an antiviral environment through the release of proinflammatory cytokines.⁹⁵ A recent study by Yao *et al.* propose that early antiviral innate responses in the genital mucosa of HIV-FSW individuals may be an important correlate of the HIV-FSW semi-resistant phenotype.⁹⁶ Yao and colleagues suggest that elevated levels of epithelial TLRs 3 and 7 may limit early HIV infection by sensing viral dsRNA and ssRNA at the portal of entry, while other immune receptors are downregulated to minimize inflammatory responses.

Additionally, mucosal surfaces are rich in diverse components belonging to the innate immune system. Soluble components of the innate immunity, including mucins, antiproteases, and antimicrobial peptides possess antimicrobial activity and are scattered within the FGT.^{97,98} For example, serine protease inhibitors (i.e. serpins and elafin) display *in vitro* anti-HIV activity and they are associated with relative HIV resistance in the genital mucosa of HIV-FSW individuals.⁹⁷⁻⁹⁹ Extensively studied host cells restriction factors including Apolipoprotein B mRNA editing enzyme catalytic, polypeptide like 3 (APOBEC 3) protein, Tripartite Motif (TRIM)5 α , tetherin and others can interfere with viral replication in

infected cells, however HIV has developed several mechanisms to abolish their anti-viral activity.¹⁰⁰

Natural killer (NK) cells are innate immune effector cells with a potent cytolytic potential. NK cells are present in all genital tissues, however they are more predominant in the upper, as compared to the lower FGT.^{55,101} NK cells are suggested to mediate early control of HIV infection¹⁰² and studies from several HESN cohorts, including African FSW cohorts, have shown an association between the functional NK cells responses in circulation and protection against HIV infection.^{103,104} It is however unclear whether the NK cell-mediated responses in the FGT mucosa are reflective of the HIV resistant phenotype.

Apart from establishing rapid antiviral immune responses, the innate immune cells also activate adaptive immune responses.⁹⁵ DCs together with other antigen presenting cells (APCs), (e.g. macrophages and B cells), take up and process antigens and thereafter migrate to nearby secondary lymphoid organs. Once in the lymphoid compartments, APCs present foreign antigenic peptides to naïve CD4⁺ and CD8⁺ T cells in major histocompatibility complex (MHC) class I and II dependent manner, respectively. Antigen-experienced T cells acquire an effector phenotype and can then migrate to the site of infection.¹⁰⁰

1.6.2. MAIT cells

Mucosal-associated invariant T cells (MAIT) are a recently described innate-like T cell population, which is present in various mucosal compartments as well as in liver and peripheral blood.¹⁰⁵⁻¹⁰⁷ Human MAIT cells express a semi-invariant T cell receptor (TCR), including Vα7.2 coupled with restricted Jα segments and limited Vβ repertoires, as well as high levels of C-type lectin CD161 and the interleukin (IL)-18 receptor α-subunit (IL-18Rα).^{106,108-110} In contrast to conventional T cells, MAIT cells recognize microbial B2 vitamin metabolites presented via the evolutionarily conserved MHC I-related (MR) 1 molecule.^{105,110-112} Additionally, some studies indicate that MAIT cells can be activated in presence of inflammatory and homeostatic cytokines, including IL-7, IL-12, IL-15 and IL-18 in an antigen and MR1-independent fashion.¹¹³⁻¹¹⁶ Mature MAIT cells display a distinct transcriptional profile, expressing both classical effector T cell transcription factors T-bet and Eomes, as well as promyelocytic leukemia zinc finger (PLZF) and retinoic acid-related orphan receptor γt (RORγt) transcription factors.^{106,107,113} This transcriptional phenotype justifies the ability of MAIT cells to produce various cytokines, including IFN-γ, TNF, IL-17, and IL-22.^{107,110,117} Mature MAIT cells exhibit effector memory phenotype and possess high cytolytic capacity. It has been shown that human MAIT cells can kill *Escherichia coli* (*E.coli*) infected APCs as well as efficiently lyse *Shigella flexneri* infected epithelial cells.^{116,118} Furthermore, Le Borhis and colleagues demonstrated that murine MAIT cells could respond to a variety of bacterial and fungal species and were protective against *E. coli* and *Mycobacterium abscessus* microbes.¹¹⁰ Likewise, human MAIT cells can also be activated by *E.coli* and are suggested to play a protective role in mycobacterial infections.^{110,119}

While it is suggested that MAIT cells do not become activated by viruses, functional impairment of circulating MAIT cells has however been observed in HIV-infected

individuals.^{110,113} Furthermore, MAIT cells have been shown to be reduced in blood of HIV-infected patients, however relatively preserved in their mucosal compartments.^{120,121} Thus suggesting that MAIT cells are affected by HIV.

1.6.3. Humoral immunity

The humoral immune responses belong to the arm of adaptive immunity, and primarily consist of immunoglobulin (Ig) secreting B cells. Humoral immune responses play a key role in the immune defence against a variety of pathogens, including HIV. Vaccine-induced broadly neutralizing antibodies protect NHP against infection with simian-human immunodeficiency virus.¹²² Moreover, polyfunctional non-neutralizing HIV-specific antibodies are present in circulation of HIV-seropositive individuals who control HIV replication (HIV-nonprogressors).¹²³

Whereas B cells represent a minor cell population within the FGT tissues, IgG and IgA – producing plasma cells are still found in the genital mucosa. Despite the predominance of IgA in mucosal compartments, cervicovaginal secretions (CVS) are more abundant of IgG than of IgA, with IgG being partly derived from the circulation.^{55,124} Nevertheless, a recent report shows the presence of HIV-1 specific IgA in CVS of women participating in a microbicide trial and remaining HIV seronegative despite repeated exposure to HIV.¹²⁵ Moreover, previous studies have shown the presence of HIV-neutralizing IgA in cervicovaginal lavage (CVL) of HIV-FSW, suggesting its association with a reduced risk of HIV acquisition.¹²⁶ Controversially, other data indicates minimal or absent IgA responses in CVL samples obtained from HESN individuals from different cohorts.¹²⁷ The discrepancies in these studies highlight the necessity of further investigating mucosal correlates of protective humoral responses.

1.6.4. Cell-mediated immunity

Generally, cell-mediated immunity is a type of immunity mediated by T cells, classically involving CD4⁺ T helper cell responses and effector CD8⁺ T cell responses.¹⁰⁰ CD4⁺ T helper cells are crucial for activation of other immune cells and particularly for helping B cells to produce antibodies. However, CD4⁺ T cells are highly susceptible to HIV infection and therefore mainly CD8⁺ T cell effector responses will be discussed here.

A plethora of data indicates the importance of CD8⁺ T cell responses in controlling HIV infection.¹²⁸ It was early observed that the appearance of HIV-specific cytotoxic CD8⁺ T cells, after onset of the acute HIV phase, correlated with the reduction of plasma viremia.¹²⁹ These findings were later confirmed in SIV-infected NHP, where intravenous administration of anti-CD8 antibodies resulted in increased viral replication in peripheral blood as well as lymphoid and non-lymphoid tissues.¹³⁰ Additionally, highly polyfunctional and cytotoxic CD8⁺ T cells responses have been observed in blood and peripheral tissues of HIV-nonprogressors from different cohorts.¹³¹⁻¹³³ Distinct circulating CD8⁺ T cells responses have furthermore been observed in HESN individuals. HIV-specific CD8⁺ T cells from blood of

HIV-seronegative FSW from Puerto Rico had significantly lower CD38 surface expression than low risk controls, in consistence with an immune quiescent phenotype of HESN individuals.¹³⁴ Kaul and colleagues have shown that the overall magnitude of circulating cytotoxic T-lymphocyte (CTL) responses was significantly lower in HIV-FSW as compared to HIV-infected women. However, CTL responses in HIV-FSW recognized different epitopes as compared to those targeted in HIV-infected women, suggesting that CTL responses differ rather qualitatively, than quantitatively in HIV-FSW.¹³⁵

Less is known about the association between the magnitude of CD8⁺ T cell responses in the female genital mucosa and protection against HIV infection. While the presence of cytotoxic CD8⁺ T cells in the cervix of HIV-infected women has been shown,¹³⁶ these responses are suggested to be largely monofunctional and do not predict viral shedding.¹³⁷

Furthermore, HIV-specific CD8⁺ T cell responses observed in cervix do not correlate with those present in circulation in both treatment naïve and HIV-infected women receiving highly active antiretroviral therapy (HAART).^{138,139} This indicates that cervical CD8⁺ T cell responses are distinctly compartmentalized and are not predictable by their circulating counterparts.

1.7. Tissue-residing T cells

In non-chronic infections, after antigen clearance, host T cells retain the ability of “long-term” memory, which upon repeated antigenic exposure results in a large magnitude of antigen-specific T cell responses. Generally, these memory T cell subsets are defined by their homing properties and anatomic distribution. Central-memory T (T_{CM}) cells exhibit a great proliferation potential and express CCR7 and CD62L receptors, required for their recirculation through the secondary lymphoid tissues. In contrast, effector memory T (T_{EM}) cells lack the constitutive CCR7/CD62L expression and instead express mucosa homing chemokine receptors and recirculate through blood and peripheral tissues. Moreover, T_{EM} are characterized by possessing more immediate effector functions as compared to T_{CM}.¹⁴⁰

More recently, studies have identified another memory subset of T cells, having as good effector potential as T_{EM}, although displaying a non-circulating phenotype and permanently residing within the peripheral tissues (T_{RM})¹⁴¹ (**Figure 5**). T_{RM} is a distinct memory population and display a unique transcriptional profile, which is different from the circulating memory T cells.^{142,143} T_{RM} cells are found both in murine and human peripheral tissues where they preferentially localize within the epithelial compartments.¹⁴⁴⁻¹⁴⁶ *Bona fide* T_{RM} cells can be defined by the expression of α E(CD103) β 7 integrin together with CD69. CD103 interaction with the tight junction protein E-cadherin mediates T_{RM} cell retention within the epithelial compartment, while the CD69-mediated inhibition of sphingosine 1-phosphate receptor 1 hinders cell egress from the tissues.¹⁴¹ However, recent data indicates that even CD103⁻ cells display a non-circulating T_{RM} phenotype, suggesting involvement of other mechanisms providing T_{RM} cell retention in tissues.^{141,147} The formation and establishment of the T_{RM} cell population are mediated by the local tissue environment, in the presence of TGF-

β cytokine.^{142,147,148} $CD8^+$ T_{RM} cells have been more extensively characterized than $CD4^+$ T_{RM} , although the majority of T_{RM} cells in human skin are $CD4^+$ T cells.¹⁴⁷

Different studies have shown that establishment of effector $CD8^+$ T_{RM} responses in peripheral sites upon vaccination is strongly associated with the control of viral infections, including Influenza, HSV and HPV.¹⁴⁹⁻¹⁵¹ For example, the establishment of HSV-specific $CD8^+$ T_{RM} responses in the mice FGT mucosa, via recruitment of antigen-specific cells from circulation, fully protected these mice against lethal challenge with HSV-2.¹⁵⁰ Moreover, in humans, vaccine-induced HPV responses have been shown to be more pronounced in cervical mucosa as compared to circulating counterparts.¹⁴⁹ Hence, the sentinel role of tissue residing effector cells provides a front-line defence against invading pathogens at the tissue barriers.

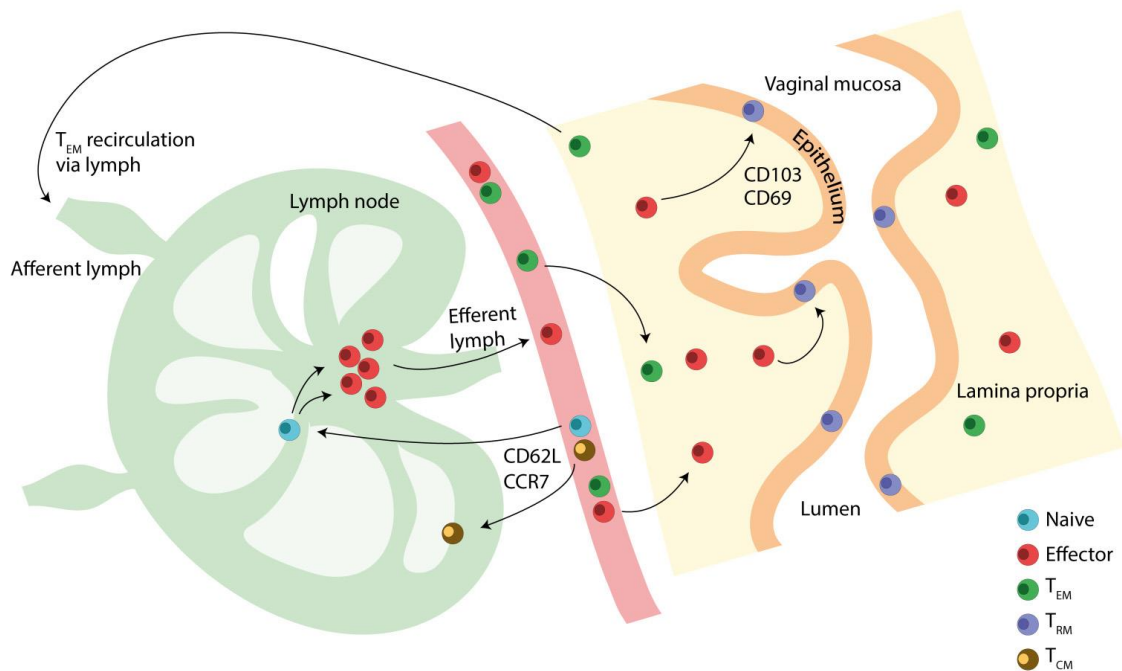


Figure 5. Schematic representation of distribution of different memory T cells. Upon antigenic stimulation, naïve T cells proliferate into effector T cells that migrate to the sites of infection. After the decline of effector phase, memory T cells recirculate through the different sites: T_{CM} cells within the blood and secondary lymphoid organs, T_{EM} between blood and non-lymphoid tissues, T_{RM} are non-circulating and are retained in the peripheral tissues. Reprinted with permission.¹⁵²

2 AIM

The main purpose of this thesis is to investigate the role of immune cells, with focus on CD8⁺ T cells, including tissue-resident CD8⁺ T cells and MAIT cells, in the FGT, the mucosal site of HIV exposure and replication.

Specific aims:

Paper I. To assess the expression of HIV target cells and levels of immune activation in the genital mucosa in HIV-infected versus uninfected women.

Paper II. To enumerate and characterize cervical CD8⁺ T cells in HIV-infected and uninfected women in relation to viral load and local viral replication.

Paper III. To enumerate and characterize CD103⁺CD8⁺ T cells in cervix and blood in HIV-infected versus uninfected women.

Paper IV. To characterize distribution and functional phenotype of MAIT cells present in the female genital mucosa and blood of healthy women.

3 MATERIALS AND METHODS

3.1. Study populations and sample collection

Samples from *Kenyatta* and *St. Göran* cohorts were used in **Paper I, II, III**, and **Paper III, IV** respectively.

The *Kenyatta* cohort: HIV seropositive (HIV⁺) and seronegative (HIV⁻) female sex workers (FSW) were recruited through the Majengo Sex Worker Clinic.⁹⁴ HIV seronegative lower risk non-sex working women (HIV⁻LR) were recruited through a Maternal Health Clinic at the Pumwani Maternity Hospital.¹⁵³ General inclusion criteria included age at least 18 years, uterus and cervix present, not actively menstruating, no symptomatic or clinically apparent cervical inflammation, willingness to undergo ectocervical biopsy collection and to abstain from vaginal sex during a healing period of two weeks. All study participants were within the same age range, were similarly distributed with regard to stages of the menstrual cycle, reported similar use of hormonal contraception, and similar numbers of pregnancies as well as sexual activity during menses.

The HIV-infected women had been HIV infected for a median of 3 years, were ART-naïve and without prior history of AIDS-defining illnesses and acute health issues. Since HIV-infected women were recruited from a sex-worker cohort, an additional group of HIV⁻ FSW was recruited to control for presence of STIs and high-risk behaviour. All FSWs enrolled were active in sex work and were comparable in terms of condom use, HSV-2 seropositivity, presence of other STIs and vaginal douching (insertion of water or water with soap in the vagina). The HIV⁺FSW included in this cohort have been followed for a median of 4 years (range 1-20) and HIV⁻FSW for a median of 7 years (range 3-19) at the date of sampling. It was furthermore established that FSW who remained HIV-seronegative despite high exposure rates, displayed a semi-resistant phenotype against HIV infection.⁹⁴ Therefore phenotype alterations of these HIV⁻FSW may be partially reflected by this rare phenotype. Paired ectocervical tissue samples, CVS and peripheral blood samples were collected from all study participants and cryopreserved until required.

The *St. Göran* cohort: Uterine tissue samples were obtained from Swedish women who underwent hysterectomy for nonmalignant and noninflammatory conditions (heavy menstrual bleeding and/or benign myoma) at the St. Göran Hospital, Stockholm. Exclusion criteria were: positivity to human papilloma virus (HPV), clinical symptoms of sexually transmitted infections during the 3 months prior to surgery and/or systemic immunosuppressive therapy. Fresh genital tissue samples were immediately transported to our laboratory. While the majority of the specimens were enzymatically digested into single cell suspension, one or two tissue blocks were snap-frozen immediately after dissection and cryopreserved at -80°C.

Blood donors: Peripheral blood samples, used in **Paper IV**, were collected from healthy Swedish women who were in the same age range as the women who underwent

hysterectomy. Blood donors were recruited at the Blood Transfusion Clinic at the Karolinska University Hospital Huddinge, Stockholm.

3.2. Ethical considerations

All study participants were provided with written and oral information about the studies. Written informed consent was obtained from all individuals in accordance with the Declaration of Helsinki. All studies were reviewed and approved by the Regional Ethical Review Board of Stockholm. Additionally, studies I, II and III were approved by the research ethics boards at Kenyatta National Hospital (Nairobi, Kenya) and University of Manitoba (Winnipeg, Canada). Our collaborators from the University of Manitoba have been involved in research studies in the Nairobi area for nearly 30 years. They have established a strong community outreach program, including regular information meetings for study volunteers and program peer leaders. All participants were provided with HIV/STI prevention counseling, male and female condoms, family planning services, treatment of STIs, medical care for acute and chronic illnesses, access to adequate diagnostic testing and referral for specialist consultant and/or hospitalization at Kenyatta National Hospital if needed.

In our studies we use ectocervical tissue biopsies obtained from the FSW. Hence, invasive sampling of the ectocervical biopsies may raise concerns regarding the increased HIV susceptibility among sex workers. Therefore, all study participants, who underwent sampling procedure, were asked to abstain from vaginal sex for two weeks after the procedure and received monetary compensation equivalent to the expected lost income. Moreover, the cervical biopsy sampling method has been evaluated and considered to be a safe and well-tolerated.¹⁵⁴

3.3. Methods

3.3.1. Quantitative real-time PCR

mRNA expression of the genes of interests in the ectocervical biopsies was assessed with quantitative real time PCR (qPCR) as described previously.¹⁵⁵ RNA was extracted from the ectocervical biopsies, stored in RNAlater solution, with a commercially available RNeasy kit (Qiagen), according to the manufacturer's protocol. RNA was further converted into complementary (c) DNA by reverse transcriptase enzyme. cDNA of the genes of interest (targets) was amplified, detected and quantified by the ABI PRISM 7700 system. Amplification of ubiquitin C (UBC) was used as an endogenous control. Ct values for target cDNA were normalized to UBC and fold change of the target genes was calculated as $2^{-\Delta\text{CT}}$.

3.3.2. Cell isolation from tissues

Genital tissue samples were collected by a pathologist immediately after the hysterectomy was performed and thereafter transported to our laboratory. Samples were maintained in ice-cold medium supplemented with antibiotics and processed within 24 hours of surgery. Fresh genital tissue samples were dissected into distinct anatomical compartments and enzymatically digested into single cell suspension as previously described.^{156,157} At least 1 cm² of mucosa (approximately 500 mg wet weight) was used for downstream applications. Enzymatically digested tissues were further mechanically disrupted. Obtained cell suspensions were passed through a cell strainer and washed in phosphate-buffered saline (PBS).

3.3.3. Flow Cytometry

The immune phenotype of single cells was assessed by Flow Cytometry as previously described.³⁷ The working principle of Flow cytometry is based on the “fluorescent antibody-cell” complex differential reaction to light. In a stream of fluid, cells bound to the fluorescently labelled antibodies pass through a laser beam one at a time. Excited by the laser, these cells emit light at distinct wavelengths, allowing to assess their properties at the single cell level.

Peripheral blood mononuclear cells (PBMCs) as well as mononuclear cells (MNCs) isolated from the genital tissues were labelled with monoclonal antibodies conjugated to fluorescent dyes, fixed and further acquired on the Flow Cytometer. In order to assess the expression of surface markers as well as cytokines and transcription factors, extracellular and intracellular antibody stainings were performed respectively. For intracellular stainings, cells were permeabilized, to enable monoclonal antibodies to enter the cell as well as the nucleus.

Data obtained from Flow cytometry was analysed (e.g. compensation, gating analyses) with FlowJo software. Multiple cell parameters were identified using the Boolean gating approach and were further analysed with Simplified Presentation of Incredibly Complex Evaluations (SPICE) software.¹⁵⁸

3.3.4. *In situ* based imaging analysis

The main focus of this thesis is the *in situ* characterization of immune cells present in the female genital mucosa. Hence, the microscopy based methods and imaging analysis will here be discussed in detail.

While plenty of data describe the HIV-associated alterations of immune responses in blood, the immunity in mucosal compartments, including genital tissues have not been as extensively studied. The natural explanation is the logistic challenges of collecting such tissue samples from HIV-infected individuals, particularly those at high-risk of infection or from endemic areas.¹⁵⁹ Genital tissue biopsies, similar in size to punch biopsies widely used in clinics, can be safely obtained from study participants, stored for years and not require

immediate processing. Microscopy-based *in situ* methods provide the fundamental approach to analyse properties of single cells in small tissue samples.

Microscopy methods have a great visual advantage as compared to other cell based techniques. *In situ* based analysis of tissue samples allows evaluation of the exact anatomical cell localization, compartmentalization and distribution within the tissue. Furthermore, it allows the assessment of the spatial cell distribution and its proximity to other cells as well as pathogens, which is a crucial component of cell-mediated immunity.¹⁶⁰ However, microscopy-based methods are associated with certain limitations. Poor antibody availability restricts the assessment of several markers at a time, resulting in the insufficient phenotypical and functional cell analysis. Moreover, evaluation of the specificity of immune cells is restricted by availability of specific reagents. For example, antigen-specific T cells can be identified with *in situ* MHC tetramer staining, which require significant expertise and reagents.¹⁶¹ However, the major limitation is the data acquisition and analysis. Manual cell counting is subjective, time consuming and particularly unsuitable for analysis of large scale samples. In contrast, automated cell counting gives higher precision, less variation and consumes less time. However, while the automated software are often insufficient when it comes to addressing inter-individual variation of biological material, manual specimen analysis performed by an expert may overcome this obstacle.

In **Paper III**, we have used the automated image analysis software CellProfiler, which is a powerful tool for quantification of different immunological parameters in tissue sections.¹⁶²⁻¹⁶⁴ CellProfiler was used to quantify fluorescently labelled cells expressing two surface markers (e.g. CD103⁺CD8⁺) in frozen ectocervical tissue sections (**Figure 6**). As compared to **Paper I** and **Paper II**, where cells were visualized with a peroxidase-labelled streptavidin-biotin amplification method, in **Paper III** and **Paper IV**, immune cells were stained with fluorescently labelled antibodies. Immunofluorescent stainings allow the assessment of several markers on a single cell more precise as compared to immunohistochemical staining since specific excitation and emission wavelengths are used to visualize the fluorescently stained cells of interest.

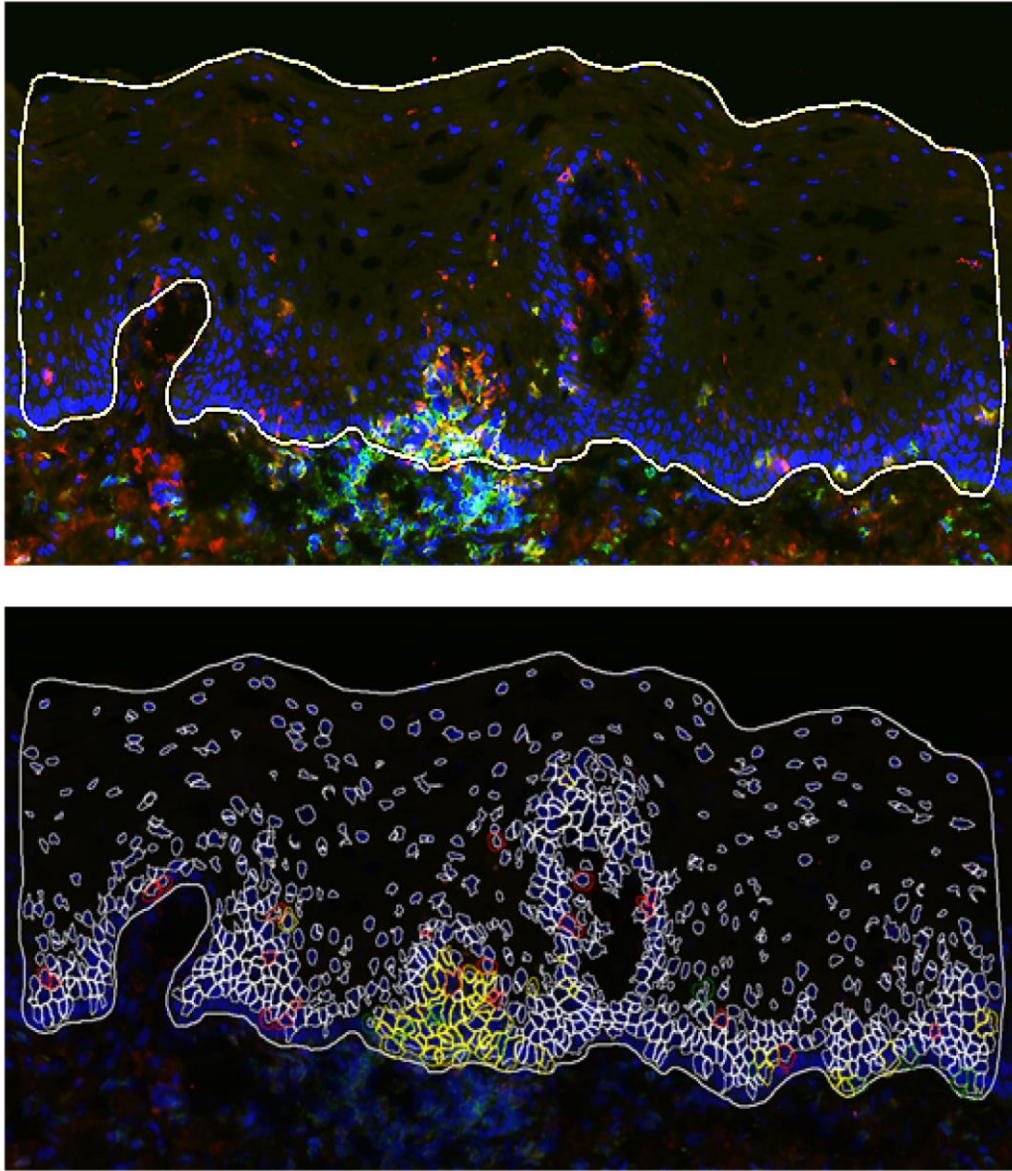


Figure 6. *In situ* analysis of immunofluorescent staining with the image analysis software CellProfiler. The upper picture shows the input image with the manually outlined region of interest (ROI; white contour). The lower picture shows the segmentation result. Nuclei within the ROI are marked with a white outline; the positively stained cells are marked with red or green outlines and double positive cells with a yellow outline.

3.4. Statistical analysis

Assessment for normality showed a Non-Gaussian distribution of values for measured parameters.

In **Papers I, II** and **III** the main objective was to assess the differences in HIV⁺FSW as compared to either HIVFSW or HIV⁻LR control groups. Hence, between the two study groups, statistical significance between continues variables was assessed using the Mann-

Whitney *U* test. Fisher's exact test was used to evaluate categorical variables. Spearman's rank correlation coefficient test was used to assess correlations.

In **Paper IV**, statistical significance between continuous variables was assessed using the Mann-Whitney *U* test for comparisons of independent samples and the Wilcoxon signed rank test for paired samples. Furthermore, in order to assess the statistical differences between the three groups, multiple comparison analysis was performed with the use of the Kruskal-Wallis test, followed by post-hoc analysis. All calculations were performed using the Prism 5.00 software and p-value of <0.05 was considered to be statistically significant.

In the SPICE analysis performed in **Papers III and IV**, statistical comparisons between two or more groups were performed using permutation t-test.¹⁵⁸

4 RESULTS AND DISCUSSION

Cell-mediated immune responses have proven to be crucial for elimination of persistent infections, including HIV. Elucidation of these immune responses at the site of exposure and infection is thus necessary for the development of effective preventive and therapeutic interventions. In this thesis I have therefore quantified and characterized immune cells residing within the FGT mucosa, with the focus on CD8⁺ T cell subsets with potential effector capacity, in HIV-infected and uninfected women.

Paper I. During HIV infection, massive CD4⁺ T cell depletion occurs at the mucosal sites, predominantly in the gastrointestinal mucosa. Moreover, HIV pathogenesis in the gut is associated with immune activation and inflammation that subsequently leads to the destruction of the gut epithelial barrier and subsequent translocation of microbial products into the circulation.^{52,165} Likewise, CD4⁺ T cell reduction as well as altered levels of other immune cells and increased immune activation has been observed in the FGT mucosa of HIV-infected women.^{29,166,167} However little is known about the expression of these cellular markers in the actual genital tissues. Thus, in this study we aimed to assess the expression of the HIV target cells (i.e. detecting the HIV-binding receptors CD4, CCR5, Langerin, DC-SIGN and MR) as well as the immune activation status in the ectocervical tissues of HIV-infected and uninfected women from the *Kenyatta cohort*.

The protein expression of immune molecules was assessed by *in situ* staining and the mRNA expression by qPCR.

The HIV-infected women reported to be infected for a median of 3 years and were ART-naïve. We found that while these women had significantly lower CD4⁺ T cell blood cell counts, they had comparable levels of CD4 expression in their ectocervix, as compared to uninfected controls (**Figure 7**). This finding suggests that CD4⁺ cells in the cervix are not depleted to the same extent as previously observed in gut. However, the CD4 expression was visualized and analysed by single staining of CD4, which does not allow us to distinguish between the different cell populations expressing CD4 (i.e. mainly CD4⁺ T cells, dendritic cells and macrophages). It is thus possible that other CD4 expressing cells (DCs, macrophages) are altered in cervical mucosa. Further assessment of gene expression for macrophage (CD68) and DC (CD11c, CD1a) markers however showed comparable mRNA expression levels in HIV-infected and uninfected women, thus indicating that expression of these cells was not altered. Chronic HIV infection is associated with high CD4⁺ T cell turnover and regeneration of the CD4⁺ T cell compartment.²⁷ It is thus plausible that the observed stable CD4⁺ expression is reflective of the “recovered” CD4⁺ T cell numbers during the chronic HIV phase. Furthermore, since HIV RNA was detected in the cervical tissues of these women (**Paper II**), one may speculate that the relative preservation of CD4⁺ cells may be involved in sustained levels of low viral replication. An interesting observation was that the HIV⁺FSW group displayed significantly lower CD4 expression in their cervix, compared to both HIV⁺FSW and HIV-LR groups. Whether, this low expression of CD4, the main HIV

receptor at the portal of HIV entry, contributes to the semi-resistant phenotype of these individuals is unclear and requires further investigations.

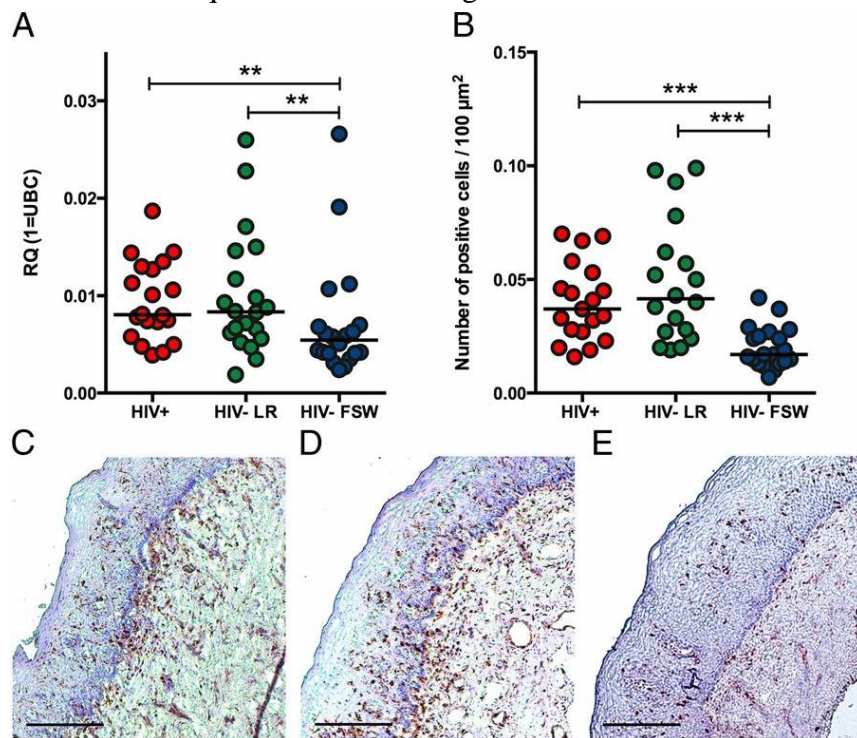


Figure 7. CD4 expression in ectocervical tissues. The graph shows the distribution of **A)** RQ (Relative Quantification (UBC=1)) of CD4 mRNA expression levels and **B)** CD4 protein levels in ectocervical tissues from HIV⁺FSW, HIV-LR and HIV-FSW. Representative picture of immunohistochemical staining showing CD4 in brown and hematoxylin in blue in the ectocervical tissues from **C)** HIV⁺FSW, **D)** HIV-LR and **E)** HIV-FSW. Reprinted with permission.⁶⁸ Copyright 2013. The American Association of Immunologists, Inc.

Moreover, the HIV-infected women had elevated levels of the HIV receptor molecules CCR5, Langerin, DC-SIGN, and MR, which may reflect pathogen-induced proinflammatory responses and influx of immune cells. In consistence with previous observations,¹⁶⁸ elevated levels of proinflammatory cytokines, including IFN- γ , TNF, IL-17 and IL-6, were here observed in the cervix of HIV-infected women (**Figure 8**). The inflammatory environment in the lower FGT may thus promote viral replication and genital shedding, which may subsequently increase the risk of sexual HIV transmission.

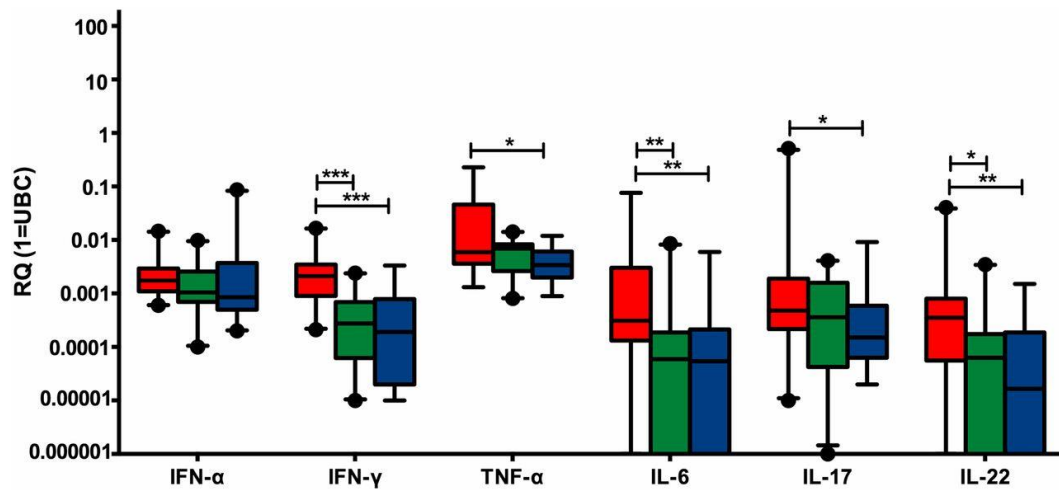


Figure 8. Quantification of cytokines. The graph shows the distribution of RQ (Relative Quantification (UBC=1)) of mRNA expression levels of cytokines in the ectocervical tissues from HIV⁺FSW (red), HIV-LR (green) and HIV-FSW (blue). Reprinted with permission.⁶⁸ Copyright 2013. The American Association of Immunologists, Inc.

Paper II. CD8⁺ T cell responses have been shown to play an essential role in controlling HIV infection.¹²⁸ However, few studies have interrogated these cells in the genital tissues, the main portal of HIV entry and the main replication site before the virus spread to lymph nodes and circulation.^{64,101,169} Cervical CD8⁺ T cell responses have been primarily assessed in cervical MNCs obtained from HIV-infected women.^{138,166,168} However, localization and spatial distribution of CD8⁺ T cells and their proximity to other cells within the tissues determines the efficiency of cell-mediated immune responses. Therefore, the main aim of this study was to enumerate and characterize CD8⁺ T cells and to discriminate the epithelial and submucosal distribution of these cells within the cervix of HIV-infected and uninfected women from the *Kenyatta cohort*. Thus, cell markers, including CD8, CD3 and HLA-DR, were assessed by *in situ* staining and by qPCR. HIV RNA expression in tissue was measured by *in situ* hybridization, while viral load in plasma and CSV (viral shedding) was measured by qPCR.

We found that, as compared to uninfected controls, HIV-infected women had significantly higher amounts of total cells, including CD3⁺, CD8⁺ and HLA-DR⁺ cells in both the epithelial and the submucosal compartments of the ectocervix. However this difference was more pronounced in the epithelial compartment. To investigate if these CD8⁺ cells were T cells, we next performed fluorescent double staining and observed that the majority of CD8 expressing cells indeed were T cells (**Figure 9**). The elevated levels of cervical CD8⁺ T cells in HIV-infected women observed here are in line with previous findings from cervical MNCs.^{166,168}

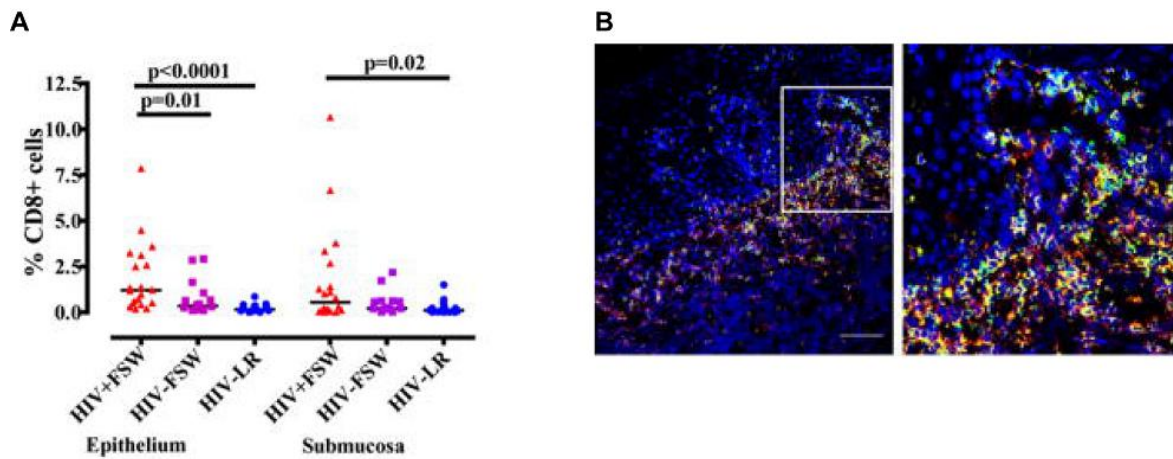


Figure 9. Enumeration and visualization of CD8⁺ cells. **A)** The graph shows the distribution and median of the percentage of CD8⁺ cells out of total tissue area in the epithelial and submucosal compartments of the ectocervix. **B)** Representative picture illustrating *in situ* staining of CD8⁺ cells (red) and CD3⁺ (green) in the ectocervical tissue from HIV⁺FSW. Double positive CD8⁺ T cells are shown in yellow. Reprinted with permission.¹⁷⁰ Copyright 2014. The American Association of Immunologists, Inc.

We could furthermore detect HIV RNA⁺ cells in the cervix of four out of the twenty HIV-infected women (**Figure 10**). Interestingly, two out of those four had low/undetectable plasma viral load (40 and 20 HIV RNA copies/ml, respectively) and undetectable viral shedding (>20 HIV RNA copies/ml). This indicates that virus levels in the genital tissues cannot be fully predicted by the virus levels present in circulation or in the vaginal secretions. Studies using NHP that were intravaginally infected with SIV have shown that SIV infection in the genital tract is highly focal, and small founder populations of infected cells (SIV RNA⁺ cells) are disperse in the FGT tissues.^{80,81} In our study, HIV RNA detection was performed in three tissues sections per individual, from 3 mm² ectocervical biopsy. It is thus possible that our results are underestimating the total levels of HIV replication in the FGT of these women, suggesting that the FGT should not be neglected as a potential viral reservoir.

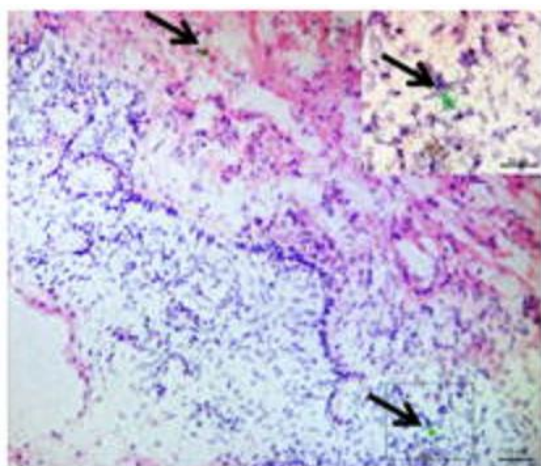


Figure 10. HIV RNA⁺ cells present in ectocervical tissue. Detection of HIV RNA⁺ cells (green silver grains indicated by the black arrows) with *in situ* hybridization in the ectocervical tissue from HIV⁺FSW. Reprinted with permission.¹⁷⁰ Copyright 2014. The American Association of Immunologists, Inc.

Furthermore, we found that the numbers of cervical CD8⁺ cells were the only immune marker that significantly correlated with plasma and cervical viral load, but not with the presence of HIV RNA, indicating the importance of CD8⁺ cells in the local HIV pathogenesis. We also assessed the levels of cellular immune activation in the cervix using HLA-DR expression as a surrogate marker for immune activation.³⁷ The HIV-infected women displayed elevated levels of HLA-DR, which furthermore correlated with the CD8 expression in their cervix. The higher levels of cellular activation observed here within the HIV-infected group are in line with our previous findings (**Paper I**). Additionally, we observed that CD8 expression significantly correlated with CD69 expression in cervix of these HIV-infected women. CD69 expression on tissue-derived cells is associated with their tissue-residing, non-circulating phenotype.¹⁴⁷ This suggests that CD8⁺ T cells present in the cervix of HIV-infected women may display a tissue-residing phenotype and requires further investigations.

Overall, our data indicates that, local immune activation and viral shedding are associated with increased levels of CD8⁺ T cells in genital mucosa. Future investigations are thus required to scrutinize the functional phenotypes of these CD8⁺ T cells and their role in the local HIV pathogenesis in genital tissues.

Paper III. In **Paper II** we hypothesised that cervical CD8⁺ T cells, particularly those localized in the epithelium displayed a tissue-residing phenotype. Thus, in study **III** we wanted to investigate if these cervical CD8⁺ T cells expressed CD103 and displayed a tissue-resident phenotype. Furthermore, we aimed to investigate if there was an association between tissues residing and circulating CD8⁺CD103⁺ T cells as previously seen,¹⁷¹ and if the numbers or phenotype of these cells were altered in HIV-infected versus uninfected women. *In situ* staining was thus performed to enumerate CD8⁺ T_{RM} cells (i.e. CD8⁺CD103⁺ cells localized within the cervical epithelium) and flow cytometry was used to characterize CD103 expressing CD8⁺ T cells in paired ectocervical biopsies and blood samples respectively, from

HIV-infected and uninfected women from the *Kenyatta cohort*. Furthermore, CD8⁺ T_{RM} phenotype was assessed by flow cytometry and *in situ* stainings in the cervical tissue samples obtained from women from the *St. Göran cohort*.

We observed that CD103⁺CD8⁺ T cell frequencies were significantly reduced in blood of HIV-infected women, as compared to uninfected controls. Circulating CD103⁺CD8⁺ T cells were enriched within the effector memory pool in HIV-infected women, however the majority of these cells displayed a transitional memory phenotype in all groups analysed. Furthermore, the CD103⁺CD8⁺ T cell population seems to be highly activated in chronic HIV infection, but not exhausted compared to the total CD8⁺ T cell pool.

Next, we investigated if the cervical CD8⁺ T_{RM} cells were altered during HIV infection. We observed that the total numbers of CD8⁺ T_{RM} cells per tissue area as well as frequencies of CD8⁺ T_{RM} cells out of total cells were significantly increased in the ectocervix of HIV-infected women as compared to uninfected women. However, the proportion of CD8⁺ T_{RM} cells out of total CD8⁺ cells was significantly reduced in HIV-infected women (**Figure 11**).

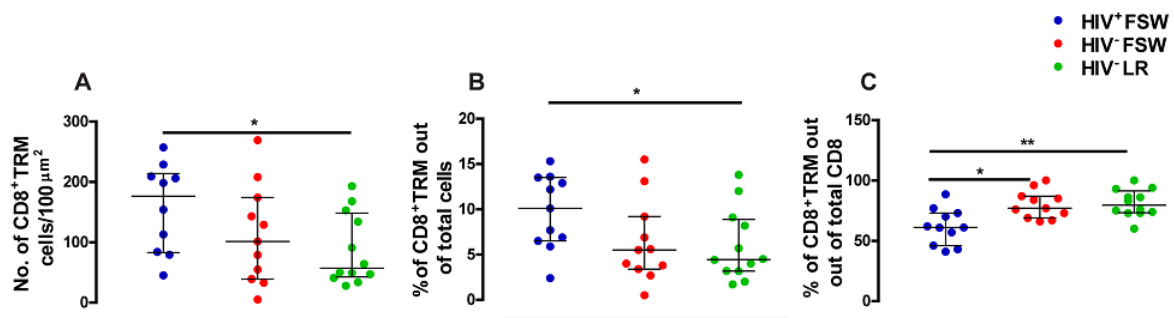


Figure 11. CD8⁺ T_{RM} cells in ectocervical epithelium. Quantification of CD103⁺CD8⁺ (CD8⁺ T_{RM}) cells in the ectocervical epithelium. The graphs show the distribution and median of **A**) the number of CD8⁺ T_{RM} cells per tissues area (100μm²), **B**) the % of CD8⁺ T_{RM} out of total cells and **C**) the % of CD8⁺ T_{RM} cells out of total CD8⁺ cells in the ectocervical tissues from HIV⁺FSW, HIV⁻FSW and HIV⁻LR.

One may speculate, that upon chronic HIV infection and persistent STIs and HIV-exposure during active sex work, CD8⁺ T cells, including CD8⁺ T_{RM} cells, may be actively recruited to/or expanded in genital mucosa.

On the other hand, the decrease in the proportion of cervical epithelial CD8⁺ T_{RM} cells out of total CD8⁺ T cells may be due to the observed infiltration/expansion of CD103⁺CD8⁺ cells in the cervical epithelium of HIV-infected women. These CD103⁺CD8⁺ cells were mainly observed in close proximity to the ectocervical basal membrane. It is possible that the CD103⁺ cells migrate further up in the epithelium, upregulate CD103 via interaction with E-cadherin and acquire a *bona fide* T_{RM} phenotype. Watanabe and colleagues have recently showed that in human skin, CD103⁺ T cells also represent a non-circulating T_{RM} phenotype, however with less effector capacity as compared to CD103⁺ TRMs.¹⁴⁷ Decreased frequencies of CD103 expressing CD8⁺ T cells and redistribution of E-cadherin have been observed in gut of HIV-

infected individuals.^{51,172} This suggests, that upon HIV infection, impaired tissue retention mechanisms may subsequently alter local CD8⁺ T cell-mediated immune surveillance. Therefore it is possible that CD103⁺CD8⁺ T cells in cervix may display an altered phenotype as compared to those expressing CD103. However, these questions could not be addressed in this cross-sectional study and further investigations of *in vivo* T cell dynamics and migration patterns are thus required.

Furthermore, we did not find a significant correlation between the frequencies of circulating and cervical CD103⁺ cells among the CD8⁺ T cell population as previously seen by Kiravu *et al.*¹⁷¹ This discrepancy may be due to differences in the techniques used as well as the source of the samples analysed. Thus, further studies are warranted to interrogate the association between tissues residing and circulating CD8⁺CD103⁺ T cells.

Nevertheless, our data further provides a valid insight in the distribution of tissue residing CD8⁺ T cells, which may have a potential capacity to eliminate incoming pathogens, such as HIV in the genital mucosa.

Paper IV. MAIT cells are a recently described subset of invariant T cells that are restricted by MHC class I-related MR1 molecule.^{105,106,112} Several studies indicate that MAIT cells display broad antimicrobial capacity by production of proinflammatory cytokines and direct killing of bacterially infected cells.^{107,116,118} While MAIT cells have been detected in various mucosal tissues,¹⁰⁷ their presence in the genital mucosa has not been investigated. Hence, in the present study, we wanted to characterize MAIT cells in the FGT tissues of healthy women and assess their phenotypic and functional properties in comparison to circulating MAIT cells. Thus, the distribution of MAIT cells within the FGT was assessed by *in situ* staining, and flow cytometry was used to enumerate as well as to phenotypically and functionally characterize MAIT cells in the FGT tissues obtained from healthy Swedish women included in the *St. Göran cohort* as well as in PBMC samples obtained from age-matched healthy Swedish women.

MAIT cells and MR1⁺ APCs were present in the upper and lower FGT, with their pronounced localization within clusters of IL-18α⁺ cells in the ectocervical epithelium. Preferential MAIT cell localization within the ectocervix may have a functional purpose, since the lower FGT is highly exposed to both pathogenic and commensal microbes. We furthermore characterized the phenotype and functional profile of MAIT cells present in the FGT and compared it with blood-derived MAIT cells. While the phenotype of MAIT cells from the FGT and blood was comparable (IL-18Rα⁺, CD127⁺, α4β7⁺, PD-1⁺, PLZF⁺, RORγt⁺, Helios⁺, Eomes⁺, T-bet⁺), their functional profile was different. Namely, stimulation with *E.coli* showed that MAIT cells from the FGT were biased towards IL-17 and IL-22 production, while blood-derived MAIT cells were more prone to produce IFN-γ, TNF and GrzB (**Figure 12**). Deeper analysis revealed that the polyfunctional profile of MAIT cells from the FGT and blood was also skewed towards Th1/Th17 and Th1 cytokine responses, respectively. The preferential IL-17 and IL-22 production by FGT-derived MAIT cells is intriguing, considering that these cytokines are essential for both antimicrobial responses and maintenance of mucosal barrier integrity.^{173,174} It is likely that production of these cytokines is modulated by the local cytokine environment, local microflora and possibly

transcriptionally regulated as it has been shown in murine MAIT cells.¹⁷⁵ Furthermore, the preferential IL-17/IL-22 production seen in the FGT as compared to blood was not limited to MAIT cells since other immune cells within the FGT also produced these cytokines.

Our data indicates that FGT-derived MAIT cells respond to bacterial stimuli in a fashion unique to FGT. In virtue of their polyfunctionality and preferential IL-17/IL-22 production, MAIT cells may act as first line of defence against incoming pathogens as well as keepers of mucosal homeostasis and barrier integrity in the genital mucosa.

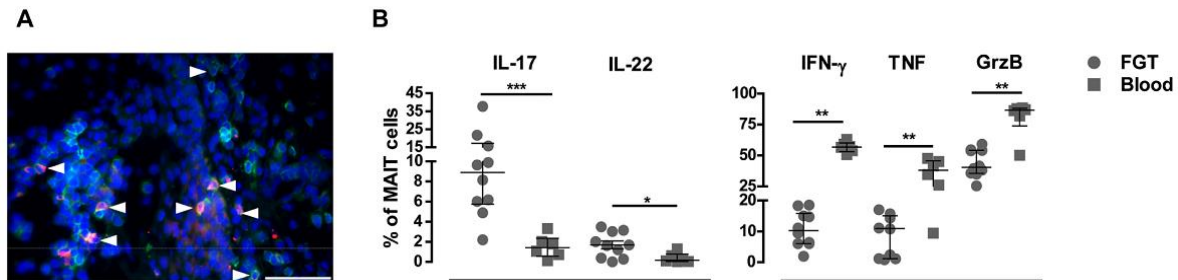


Figure 12. MAIT cells in the FGT and blood. **A)** Representative picture of immunofluorescent staining of Va7.2⁺ (red) and IL-18R α ⁺ (green) cells in the ectocervical tissue from healthy women. Double positive MAIT cells are indicated with white arrows. DAPI (blue) was used as a counterstain for visualization of cell nuclei. **B)** The graphs show the distribution and median of cytokine and GrzB production by FGT- and blood-derived MAIT cells, assessed by flow cytometry.

Moreover, the presence of IL-17 and IL-22 producing MAIT cells at the portal of HIV entry and replication site is intriguing, since a severely compromised mucosal barrier is a hallmark of HIV infection.^{52,165} Little is known about MAIT cells in HIV pathogenesis, except that these cells are not preferentially infected by HIV.¹²¹ However, levels of MAIT cells are reduced in blood and relatively preserved in mucosal compartments of HIV-infected individuals, suggesting that circulating MAIT cells may be recruited to the barrier tissues for preservation of mucosal barrier integrity.^{120,121} Given the early depletion of cervical Th17 cells, it is tempting to speculate that the presence of IL-17/IL-22 producing MAIT cells in the genital mucosa would play an important role for the preservation of the barrier integrity and subsequent prevention of the dissemination of local infections. Thus, further investigations are warranted to interrogate the role of MAIT cells in genital mucosa upon HIV infection.

5 CONCLUSIONS AND FUTURE PERSPECTIVES

During the 30 years of the HIV epidemic different vaccination approaches have been tested and all failed to achieve levels of protection that would be needed for clinical efficacy in a global perspective.¹⁷⁶ Even today, a development of an effective HIV vaccine remains to be elusive. On the other hand, preventive interventions including PrEP (up to 90 % protection in different cohorts), male circumcision (up to 60% protection) and antiviral drug administration with topical gels (CAPRISA, >50% protection in high adherers) or vaginal ring (Vaginal ring with Dapivirine, 37% protection) have shown better protection against HIV infection than the most successful HIV vaccine trial RV144 (31% protection).¹⁷⁷⁻¹⁸¹ Despite the high effectiveness of PrEP, treatment based eradication of the HIV epidemic is not economically or logistically feasible. Hence other implementations of preventive measures to stop sexual HIV transmission/acquisition at the site of exposure (genital mucosa) before establishment of systemic infection are also needed and may provide an effective approach to end the HIV epidemic.

As discussed above, the initial HIV transmission across the female genital mucosa is associated with a great vulnerability of the virus and may thus provide a “window of opportunity” for preventive interventions. However, lack of understanding of immune correlates of HIV protection at the portal of entry makes it difficult to formulate what kind of immune responses would be desirable to achieve. Furthermore, limited availability of genital tissue samples obtained from individuals infected with or exposed to HIV hamper the analysis of these responses. Therefore in this thesis we aimed to characterize cervical immune cells in a unique set of samples obtained from HIV-seropositive and seronegative FSW, at high risk of HIV infection, and lower risk women from the same geographical area.

In **Paper I** and **II** we report that HIV-infected women display high immune activation together with ongoing viral replication in their cervix that may subsequently lead to increased HIV transmission. Thus, high levels of immune activation and active viral replication may contribute to altered barrier integrity of the genital mucosa, as previously shown in gut tissues, which can subsequently lead to dissemination of local infections.^{50,165} Furthermore, we also observe that the HIV-infected women had comparable levels of CD4 expression in their ectocervix, as compared to uninfected controls. Interestingly, decreased numbers of CD4⁺ cells were observed in cervix of HIV-seronegative FSW, who display a semi-resistant to HIV infection. Taken together, this suggests, that low levels of HIV target cells and low local immune activation in genital mucosa are crucial parameters for reduced HIV acquisition and thus have to be considered in mucosal vaccination strategies.

In **Papers II** and **III** we show that HIV-infected women display elevated levels of cervical CD8⁺ cells, particularly those present in the epithelium (CD8⁺ T_{RM}). Elevated numbers of cervical CD8⁺ T cells implicate that they play an important role in local viral pathogenesis. We have not assessed the functionality and specificity of these cells in the cervix of HIV-infected women and uninfected controls. It would thus be interesting to investigate whether

these CD8⁺ T cells are beneficial for control of HIV replication and to scrutinize the phenotypic and functional alterations of these cells in HIV infection.

In SIV-infected NHP CD8⁺ T cell responses are characterized as “too little too late”, meaning that *too little* anti-viral responses occurs *too late* at the initial site of HIV infection, after the peak of viral replication.¹⁸² This suggests that optimal protection may be achieved by potent effector responses elicited at the site of exposure/initial replication, exploiting the window of viral vulnerability.¹⁵² Different studies have shown that a high magnitude of antigen-specific CD8⁺ T cell responses can indeed be induced and maintained at the portal of entry. However, the majority of these vaccination approaches have been studied in murine models and have to be further evaluated in humans. Intranasal immunisation has shown to prime long-lived HSV-2-specific CTL responses in genital-associated lymphoid tissues.¹⁸³ Furthermore, intravaginal prime-boost immunisation elicited potent genital CD8⁺ T cell responses against HSV-2 and HIV.^{184,185} Shin and colleagues have exploited a “pull and prime” vaccination approach, by first systemically priming CD8⁺ T cell responses and then initiating recruitment of these cells to the genital tissues. Importantly, pull and prime vaccination strategy did not result in either elevated levels of genital CD4⁺ T cells or mucosal inflammation.¹⁵⁰

In **Paper IV**, we showed that polyfunctional, IL-17/IL-22 biased MAIT cells are present in the FGT mucosa of healthy women. It is tempting to speculate that antimicrobial MAIT cells, at the portal of HIV entry, may be important for the preservation of the genital barrier integrity and act as a first line of defence against invading pathogens. Moreover, FGT-derived MAIT cells displayed a unique functional phenotype, distinct from circulating counterparts. This highlights the uniqueness of the FGT environment, indicating that systemic responses cannot be used as a general marker of the immunological environment.

We hypothesise that the presence of effector immune cells at the portal of HIV entry would be beneficial for the viral control at the initial phase of viral transmission. Hence, vaccine-induced potent cell-mediated immune responses could potentially eliminate founder HIV populations before viral dissemination to lymphoid tissues and establishment of systemic infection. Thus, studies described here may contribute to the knowledge needed for the development of better vaccination and/or microbicide strategies against HIV and other sexually transmitted infections.

Nevertheless, the HIV epidemic does not happen in a laboratory environment and therefore all potential intervention strategies must be implemented considering affected populations. In 2010, speaking about HIV, Michel Sidibé, Executive Director of UNAIDS said, “This epidemic unfortunately remains an epidemic of women”. In 2015, the majority of new adolescent infections globally were still represented by young women. This “feminization” of the HIV epidemic is particularly pronounced in Sub-Saharan Africa and can be associated with various social and cultural factors. Women experience more violence in their relationships, which together with their lower socioeconomic status lead to power imbalance. In these relationships, women cannot take their own decisions about safer sexual practices, including use of contraception.¹⁸⁶ From this perspective, implementation of antiviral drug administration with topical gels or vaginal rings is very valuable and benefits women of

taking their own preventive measures. Furthermore, FSW is a key population where the HIV prevalence is twelve times higher compared to the general population.

HIV transmission in women is very complex, since it affects not only their sexual partners but also their children. That being said, effective protection of women would have a great impact on the general HIV epidemic and hence should be considered a high priority globally.

6 ACKNOWLEDGEMENTS

I am sincerely grateful to everyone that has directly and indirectly contributed to this thesis. In particular, I would like to acknowledge:

Dr. Annelie Tjernlund. My supervisor. Thank you for accepting me as a PhD student and for giving me this great opportunity to gain scientific knowledge, critical thinking and grow and mature as a person. Thank you for being a great scientific mentor, your guidance and passion for science has been a great motivation during these years. Thank you for being a great supervisor, for your expertise, encouragement and support, and for always being there for me when needed at most. Thank you for all above, but mostly for being such a great person and friend. I would not be the researcher I am today without your help.

Prof. Kristina Broliden. My co-supervisor. Thank you for being such an inspirational role model, your knowledge, enthusiasm and wisdom have always been very encouraging. Thank you creating such a welcoming and friendly atmosphere in our group and for being the best travel companion in South Africa.

Maria and Frideborg: My “HIV comrades”. Thank you for all times that we spend at the conferences and for always helping out in the lab. Thank you for our endless discussions about science and life and for all fun that we had at work and “after-work”. Thank you so much for being such great colleagues, but mostly for being such great friends to me.

Taha, for your great and dedicated work with the Kenyan cohort. I am truly grateful for the opportunity of working with these unique samples. Thanks for sharing your scientific knowledge, your ability to see the “bigger picture” is very inspiring.

Andrea, for your great scientific input and sharing your excellent laboratory experience. I have learned a lot from you in the lab and also about scientific critical thinking. Thank you for always taking you time for scientific discussions, it has been very educational for me.

My “office mates”, **Asgar** and **Gökçe,** thank you for really nice scientific and non-scientific discussions during lunches and coffee breaks.

Special thanks to **Mia,** for your never ending energy and taking such a good care of all of us.

Pernilla, thanks for teaching me laboratory techniques as well as for all chats and good times!

Thanks to all **current** and **former** members of **Kristina Broliden** and **Anna Färnert** groups: **Gabriella, Samuel, Martina, Calle, Lars, Pauline** as well as **Sara, Klara, Victor** and **Manijeh** for being such wonderful colleagues.

Olle Kämpe’s group: **Åsa, Daniel, Nils** and **Frida** thanks for great scientific discussions during the Wednesday meeting.

To people who were there in the beginning of my scientific carrier, my scientific mentors **Matti Sällberg** and **Lars Frelin** as well as former co-workers and friends **Sepideh, Fredrik, Gustav, Anette, Erwin, Markus, Antony, Joana, Jenny, Halime, Babbi** and **Kajsa.**

To all our **Canadian** and **Kenyan** collaborators, as well as to **Johan, Edwin, Liv, Stanley, Carolina** and **Petter,** it is a great pleasure to work with you.

To **Folke Flam, Emilia, Axel, Klara, Tove** and everyone at St. Göran hospital who made it possible for us to set up such a great project.

Special thanks to **Dominic** and **Marcus** for a productive collaboration and for taking such a great care of me in the US☺

To our wonderful secretary **Anne Rasikari**, for always being available and helping out.

To my beloved family: **Mom** and **Dad**, thank you for everything that you did for me, for your unconditional love and support. There are no words to say how much I love you.

To my “**extended family**” for great times in Tällberg and for lovely family dinners. Special thanks for my mother in law **Berit**, for her invaluable input with “language corrections” during thesis writing.

To all my **friends**. For making my life so full of joy, happiness and love. I am grateful to have you all in my life.

To my husband **Robert**. Thanks for always being there for me. I don’t know what I would have done without your love and support. I am so grateful for having you in my life and I love you very much.

And last but not least, I would like to express my sincere appreciation to all **study participants** in Sweden and Kenya. You made this all possible and my research is dedicated to you.

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